EFFECTS OF TEMPERATURE ACCLIMATION ON SOME ASPECTS OF CARBOHYDRATE METABOLISM IN DECAPOD CRUSTACEA [1]

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Temperature is one of the environmental factors to which the organism must adjust if it is to exist successfully in its habitat. In temperate-zone forms the severe temperature extremes of mid-winter and late summer may result in a shift of metabolic processes, tending to compensate for these extremes. However, tropical forms living in a relatively constant thermal environment do not have to contend with such extremes and their metabolic processes may not show a compensatory shift (Vernberg, 1962). Thus the temperature effects on an animal may be reflected in its physiology. Past investigations of temperature effects on populations have concentrated upon comparisons of rate functions such as oxygen consumption, ciliary activity, heart beat, and thermal limits of tissues and/or whole organisms (Bullock, 1955). It is to be expected that the ability to exist at an environmental temperature is expressed in the physiological and biochemical responses of the animal. The nature of these responses to temperature may vary with species or stage of the life cycle. Thus, not only is there a variation in the rate function of metabolic change with temperature adaptation, but the nature of the metabolic reaction or pathway may be altered (Ekberg, 1958; Hochachka and Hayes, 1962). The present research has been concerned with possible variations in carbohydrate metabolism with temperature acclimation. Several different species of crabs have been studied, using physiological parameters such as blood glucose, the total reducing sugar in the blood and hepato-pancreas glycogen levels. Also included was a qualitative analysis of blood carbohydrates using chromatographic techniques.

Material and Methods

Glucose oxidase (Huggett and Nixon, 1957) was used for the determination of blood glucose and the classic Folin-Wu method (1920) for the total reducing sugars. Glycogen was determined by the phenol-sulfuric acid method of Montgomery (1957). Chromatography of blood carbohydrates was done with ascending, descending and two-dimensional flow on Whatman #1 and #3 filter paper. Various solvents were used, with the best resolution obtained with n-butanol, ethyl alcohol, acetic acid and water in an 8:2:1:3 mixture by volume. The papers were washed in the solvent system prior to spotting the unknown. Sprays for the analysis of unknown carbohydrates included silver nitrate and sodium hydroxide,

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aniline hydrogen phthalate, iodine vapor, P-anisidine HCl and triphenyl-tetrazolium chloride. Blood samples were collected from different sites in the various species of crabs used: *Uca pugilator*, *U. minax* and *U. pugnax* were sampled in the second segment proximal to the cheliped; while *Callinectes sapidus* were sampled from the sinus at the base of the fifth pereiopod and for *Cancer irroratus*, *Libinia emarginata*, *Panopeus herbstii* and *Menippe mercenaria*, directly from the heart. Males only were used for analysis to reduce the possible effects of the hormonal factors associated with the reproductive cycle in the female (Dean and Vernberg,

CHROMATOGRAPHIC PRESENCE (+) OR ABSENCE (-) OF CARBOHYDRATES IN BLOOD

	Maltotetraose Maltotriose Maltose Glucose	Galactose	Mannose	Fucose	Galactan Derivative
Callinectes sapidus	+	+	±	-	+
Cancer irroratus	+	+	-	-	+
Libinia emarginata	+	+	±	±	+
Menippe mercenaria	+	+	+	+	+
Panopeus herbstii	+	+	-	-	+
Uca minax (Early Spring)	+	+	-	-	+
Uca minax (Autumn)	+	+	±	±	+
Uca minax (10°), (18°), (28°)	+	+	-	-	+
Uca pugilator (4°), (18°), (30°)	+	+	-	-	+
Uca pugilator (Early Spring)	+	+	+	-	+
Uca pugilator (Autumn)	+	+	+	-	+
Cancer magister[1] and Hemigrapsus nudus	+	±	-	±	-
Orconectes virilis[2]					

1 Meenakshi and Scheer

2 McWhinnie and Saller

TABLE I

1964). For chromatography, the blood was deproteinized by heating in a boiling-water bath for one minute and the supernatant fraction obtained after centrifugation was desalted (Dowex AG-501-X8); the resulting effluent was taken to dryness, dissolved in water to give a concentration equivalent to about 20 micrograms per microliter of dried material and standards were run with each unknown (McWhinnie and Saller, 1960). R_f and R_g values were calculated for comparison with values obtained by other workers. In acclimation experiments, other than the chromatography, *Uca pugilator* were used. These animals were acclimated to a given temperature for a minimum of three weeks. They were kept under a uniform 14 hours light and 10 hours dark photoperiod and the water was main-

tained at a constant salinity of 30‰ and changed every 36 hours on an 8 AM, 8 PM, 8 AM schedule. The diet consisted of Clark's fish pellets. This maintenance procedure resulted in a very low mortality in the laboratory animals. For the eyestalk experiments, the eyestalks were removed at their base and the wound closed with a cold cauterizer.

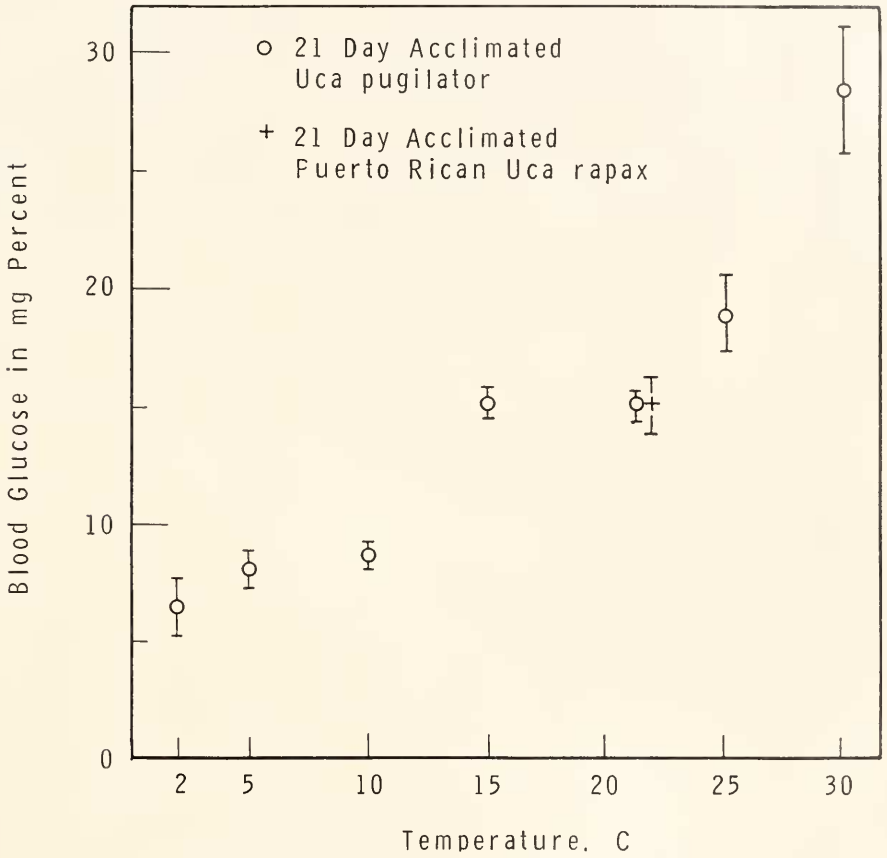

FIGURE 1. Blood glucose in acclimated crabs.

RESULTS AND DISCUSSION

The qualitative chromatography of blood carbohydrates, as seen in Table I, in seven species of crabs gave similar results. All species have maltotetraose, maltotriose, maltose, galactose, glucose and a galactan derivative. Some differences occur in the appearance of mannose and fucose in some species. Mannose is present in *Menippe* and early-spring *Uca pugilator* and possibly in *Libinia*, autumn *Uca minax* and *Callinectes*. Fucose is definitely present in *Menippe* and possibly in *Libinia* and autumn *Uca minax*. Glucose-6-phosphate is known to be present in the blood of these crabs from other work done in this laboratory. These results compare favorably with those obtained by Hu (1958) for the shore crab,

Hemigrapsus nudus and *Cancer,* and the work of McWhinnie and Saller (1960) on the fresh-water crayfish, *Orconectes.* Fairbairn (1958), using disc chromatography, has demonstrated trehalose in the tissues of several crustaceans. Using colorimetric techniques, we detected trace amounts of trehalose in the blood of several species and a higher amount was found in the blood of *Libinia.* However, these results are quite variable. Samples of blood of three species of *Uca* acclimated to different temperatures have been analyzed by chromatography. No qualitative differences could be seen in the blood carbohydrates.

Results of blood glucose and total reducing sugar levels of crabs acclimated to different temperatures would indicate that the concentration of glucose in blood is depressed at the lower temperatures (Fig. 1). A minimum of 15 individual samples was used in the determination of each point, and the figures show the mean value and standard error. Blood glucose is usually 20–25% of the total reducing sugar value of the blood. This ratio does not seem to vary significantly with

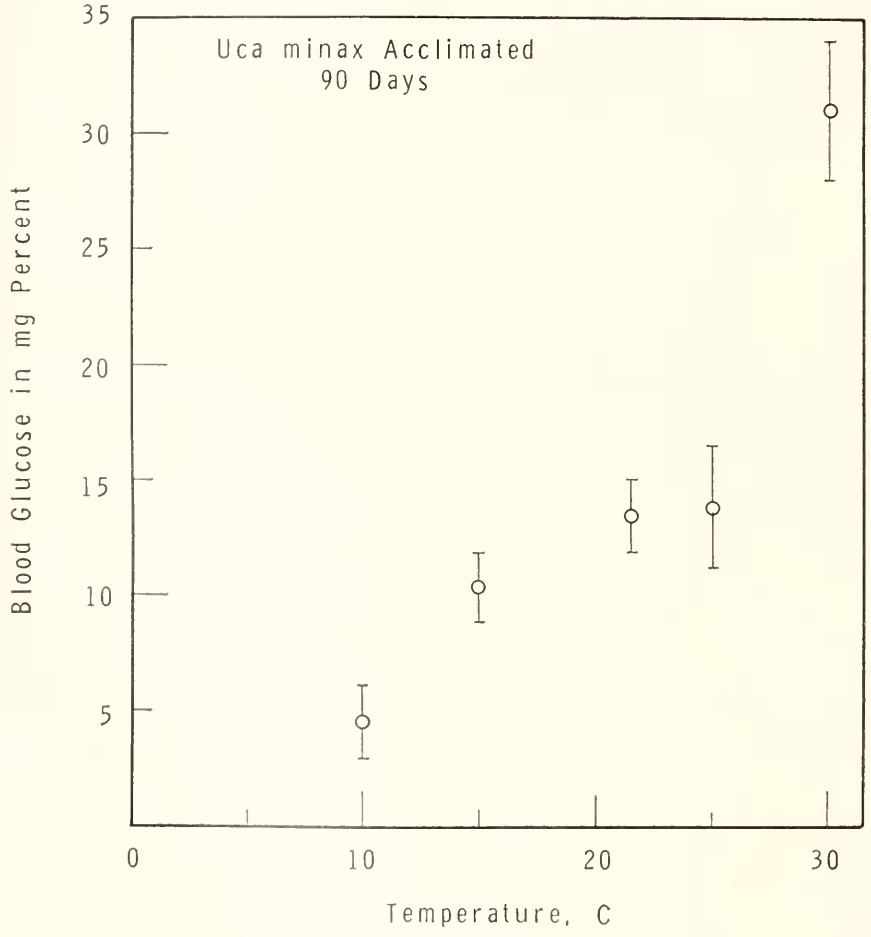

FIGURE 2. Blood glucose in acclimated crabs.

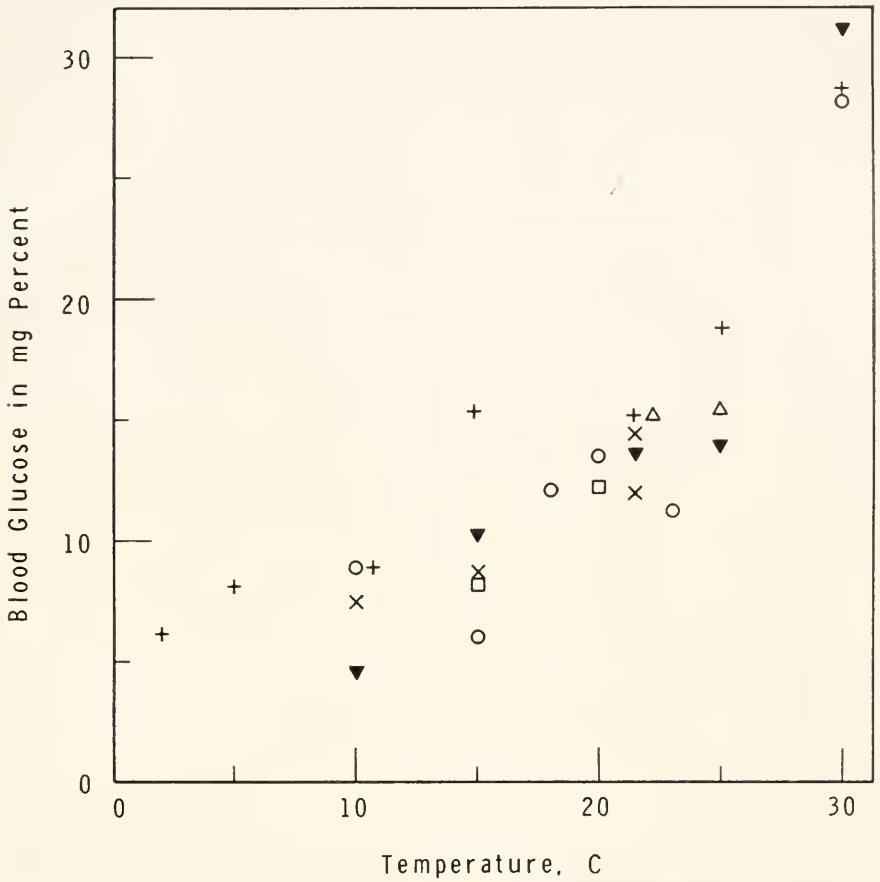

FIGURE 3. Blood glucose in crabs (X—*U. pugilator*—field animals, +—*U. pugilator*—21 day acclimation, ○—*U. minax*—21 day acclimation, ▼—*U. minax*—90 day acclimation, △—*U. rapax*, □—*U. pugnax*).

acclimation. Also, a tropical species (*Uca rapax*), acclimated at the same temperature as temperate species, has similar blood glucose values. Apparently laboratory acclimation had results similar to natural field conditions because 2° laboratory-acclimated *U. pugilator* had a low blood glucose value, as did the newly emerged crabs in early March. Short periods of acclimation to higher temperatures resulted in a higher blood glucose level, and long-term acclimation to high temperature, as in *U. minax*, showed an even more marked increase. However, *U. minax* shows much the same response as *U. pugilator* (Fig. 2). Long-term acclimation, in this case three months, resulted in a trend to temperature-dependent blood glucose values. The level for field animals emerging early in the spring and acclimating animals followed the patterns seen in *U. pugilator*.

Uca pugnax, a temperate-zone form, fits the range of blood glucose values for the other species (Fig. 3) and *U. rapax*, which is a tropical species, is also in the same general range. Thus, it may be seen that there is a general trend to low

blood glucose at low temperature and an upward trend at higher temperatures. The data indicate a plateau for blood glucose around the optimum temperature range of the animal, which is a pattern similar to that seen in respiration experiments (Vernberg, 1959).

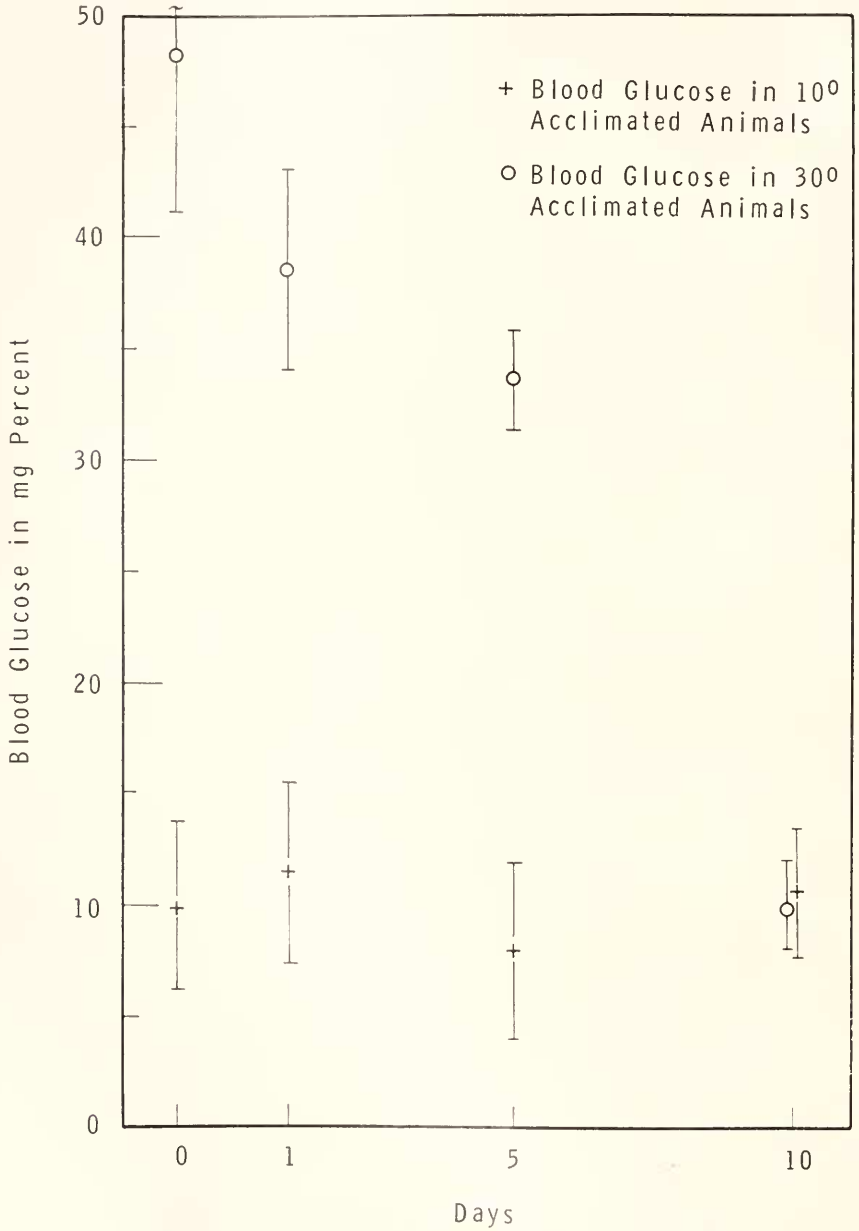

FIGURE 4. Effect of fasting on blood glucose in *U. pugilator*.

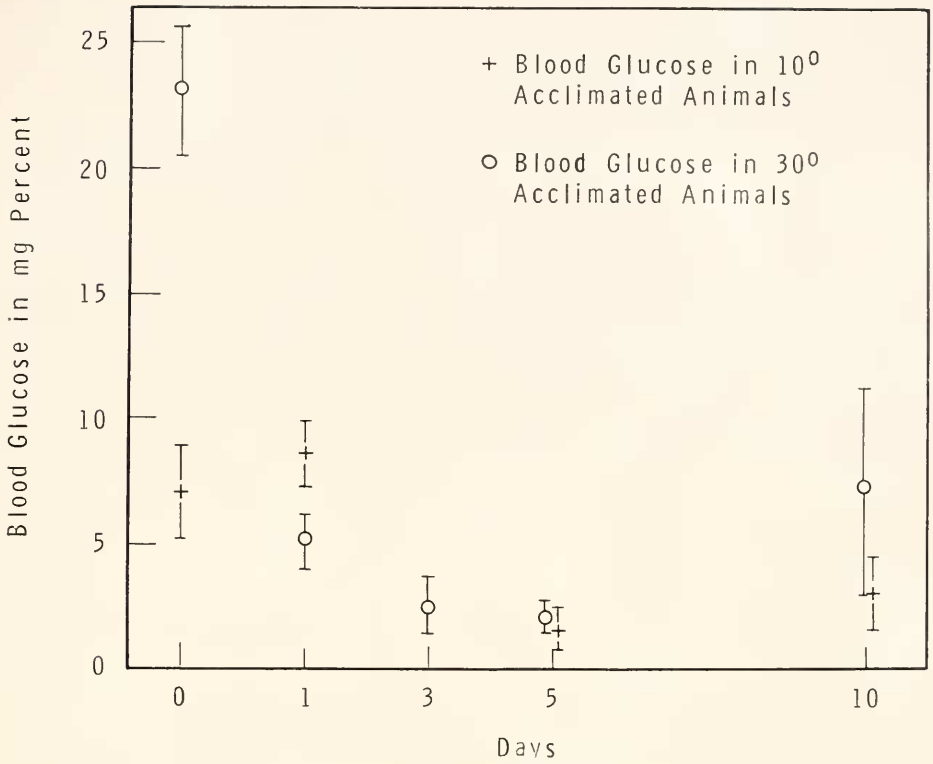

FIGURE 5. Effect of eyestalk removal on blood glucose in *U. pugilator*.

Qualitatively there is no major change in blood carbohydrates with acclimation. However, there may be quantitative differences, as shown by the lower blood glucose values obtained with animals acclimated to low temperatures.

We were interested also in the effects of diet and hormones in relationship to the carbohydrate metabolism of the animal. Crabs were well acclimated for 21 days at 10° and 30°. They were then fasted and sampled on days 0, 1, 5 and 10. Unlike *Libinia*, which shows no change in blood sugars with fasting or eyestalk removal (Kleinholz and Little, 1949), it may be seen in Figure 4 that the blood glucose in the fasting 30° *U. pugilator* decreased consistently with time while the fasted 10° animals were fairly constant. The hepato-pancreas glycogen levels followed this same pattern.

Eyestalk removal during the intermolt stage will induce molting in the crab except at lower temperatures where temperature acts as a molt inhibitor (Passano, 1960). During ecdysis, a period of high physiological activity, several dramatic changes in the carbohydrate metabolism occur. To induce molting, eyestalks were removed from 10° and 30° acclimated *U. pugilator* fed *ad libitum*. Samples were taken on days 0, 1, 3, 5 and 10. Under these conditions, which initiate the molt sequence of events, there is an extremely rapid drop in the blood glucose level of the 30° animals (Fig. 5). This remains at a low level for a period of time and then begins a slow increase. However, the 10° animals did not show this change

in blood glucose level, did not molt or initiate proecdysis, and had a high mortality rate. There is no significant change in the hepato-pancreas glycogen values in the 10° animals but the 30° crabs showed a typical buildup in hepato-pancreas glycogen as molt approaches, and rapid decline with ecdysis.

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Summary and Conclusions

The preceding work on carbohydrate metabolism in *Uca* would suggest the following:

First, with temperature acclimation there is no qualitative shift in carbohydrate metabolic pathways, but rather there may be quantitative variations. Second, at low temperature the animal reduces its energy output to a minimal level. This may be related to the energy demands of the molt cycle. It would seem that even though sufficient carbohydrate reserves are present at low temperature, there may be variations in hormone levels which would affect the molt cycle. These physiological characteristics correlate well with field observations and the general ecology of the fiddler crab. However, generalities cannot be made for all Crustacea as there are obvious differences between genera.

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